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Determining the relative sensitivity of benthic diatoms to atrazine using rapid toxicity testing: A novel method

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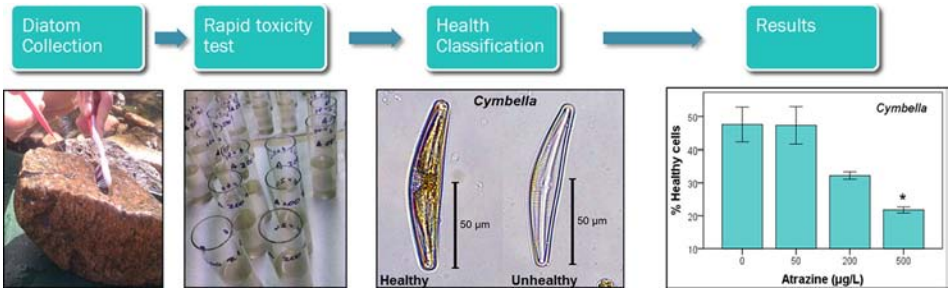
HIGHLIGHTS

- A novel method is described for the rapid toxicity testing of benthic diatoms to herbicides.
- Atrazine sensitivity is determined for individual diatom taxa from a natural benthic community.
- Atrazine concentration had a negative effect on the health of diatom cells in the sensitive taxa.
- Effects on community structure were evident after 48 h of atrazine exposure.

GRAPHICAL ABSTRACT



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ABSTRACT

Herbicides pose a potential threat to aquatic ecosystems, especially to phototrophic organisms such as benthic diatoms. Benthic diatoms may be a valuable indicator of the toxic impacts of herbicides in aquatic systems. However, this requires information on the herbicide sensitivity of a wide range of freshwater benthic diatom taxa. Unfortunately this information is only available for a limited number of species as current methods of developing new algae toxicity tests on individual taxa are lengthy and costly. To address this issue, we developed a new rapid toxicity test method to test natural benthic communities, from which the relative herbicide sensitivity of many individual taxa can be derived. This involved the collection of natural benthic communities from rocks *in situ*, which were placed directly into laboratory toxicity tests. Sensitivity data for several diatom genera in a 48 hour exposure toxicity test were produced, without the need for cultures or multiple site visits. After exposure to the highest treatment of atrazine (500 µg L^{-1}) there were significant declines of healthy cells in the most sensitive genera: *Gomphonema* declined by 74%, *Amphora* by 62%, *Cymbella* by 54% and *Ulnaria* by 34% compared to control levels. In contrast, the genera, *Eunotia*, *Achnanthidium* and *Navicula*, had no statistically significant decline in cell health. This method can identify the diatom taxa most at risk of herbicide toxicity within the natural benthic diatom community. The rapid toxicity testing method presented is a simple and effective method to obtain sensitivity data for multiple taxa within a natural benthic diatom community in a relatively short period of time.

1. Introduction

Herbicide contamination of freshwater ecosystems poses a potential threat to primary producers, such as benthic diatoms, and they may be a valuable indicator community for toxic impacts (DeLorenzo et al., 2001). Benthic diatoms are ubiquitous and respond rapidly to environmental conditions; therefore, changes in community composition due to herbicide toxicity may reflect past herbicide concentrations (Burns and Ryder, 2001; Villeneuve et al., 2011). Herbicide exposure in streams typically occurs as pulses associated with diffuse agricultural runoff, and as a result, routine (i.e. calendar based) sampling of herbicides will most likely underestimate herbicide concentration and thus toxicity (Davis

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et al., in press). In order to address this, chemical monitoring needs to include event based sampling after rainfall and during floods to estimate the peak concentration of herbicides and/or include the use of passive samplers to estimate the average concentration. However, these measures require multiple site visits, increasing the cost of monitoring. Furthermore, with any chemical monitoring there is uncertainty as to the ecological risk of the chemicals observed and the chemicals detected may not be the entire suite of chemicals present in the field (Magnusson et al., 2008). Consequently, there is a need for biomonitoring tools that give an integrated response to chemicals over time, and freshwater benthic diatoms may be a cost effective and ecologically relevant solution for herbicides (Debenest et al., 2009; Morin et al., 2009).

Linking field effects to any one particular stressor in the environment can be problematic due to the range of variables that can alter community structure and the influence of multiple stressors (Morin et al., 2009; Schäfer et al., 2007). However, Schäfer et al. (2011a) proposed a conceptual model for trait based biomonitoring indices that link exposure to a specific stressor with community composition changes in the field, such as the SPEcies At Risk (SPEAR) index (Liess and Ohe, 2005). The $SPEAR_{pesticides}$ index has been developed using macroinvertebrates to describe changes in the proportion of sensitive taxa within a community, relative to the intensity of pesticide stress (Liess and Ohe, 2005). The key trait used in $SPEAR_{pesticides}$ is the sensitivity of macroinvertebrate taxa to organic toxicants (Liess and Ohe, 2005; Schäfer et al., 2007). $SPEAR_{pesicides}$ has been used successfully in Europe and also in Southeast Australia, to link pesticide exposure (mostly insecticides and fungicides) to field effects (Liess et al., 2008; Schäfer et al., 2011b). However, $SPEAR_{pesticides}$ is less effective at predicting herbicide toxicity as it uses macroinvertebrates as indicators which respond more strongly to insecticides and fungicides (Schäfer et al., 2011c). Benthic diatoms may be a more suitable indicator community to assess herbicide toxicity, especially photosystem II inhibitors (PSII), as their phytotoxic effects have been established (Debenest et al., 2010; Magnusson et al., 2010, 2012).

The principle impediment to developing a biomonitoring index for herbicides, based on the community composition of diatoms (or other primary producers) is lack of information on how particular taxa respond to herbicides (Culp et al., 2011; Morin et al., 2009; Roubeix et al., 2011). Although some information exists on the toxicity of herbicides to a few freshwater benthic diatom species (Debenest et al., 2009; Larras et al., 2012; Magnusson et al., 2010; Tang et al., 1997), for any particular region, there are very few taxa with herbicide sensitivity data (Magnusson et al., 2012). This is in part due to the time constraints and costs of current standard toxicity tests which involve the use of single species cultures to determine individual sensitivities. Cultures of most species are unavailable and obtaining sensitivity data for numerous species by standard toxicity testing methods would be very time consuming. A new method that can produce sensitivity data for a number of local taxa in a relatively short period of time would be ideal for obtaining the required data for a traits-based monitoring index that can detect herbicide toxicity in rivers (Culp et al., 2011). We followed the rapid toxicity approach which aims to determine herbicide toxicity to multiple taxa from a multispecies community in a relatively short period of time (Hickey et al., 2009; Kefford et al., 2005). Other studies either use single species cultures to produce this sensitivity data for individual taxa (Larras et al., 2013; Magnusson et al., 2010; Roubeix et al., 2011), or use community level measures of health such as photosynthetic inhibition that cannot determine which taxa within the community are contributing to the sensitivity (Magnusson et al., 2012; Proia et al., 2011; Prosser et al., 2013).

This paper establishes a new method to determine the relative herbicide sensitivity of field derived freshwater benthic diatom taxa using rapid toxicity tests. These tests aim to produce relative sensitivity data for several freshwater diatom taxa in one 48 hour test (see Kefford et al., 2003). The current study utilises a new approach to place benthic diatoms collected *in situ* directly into rapid toxicity tests that can determine the relative sensitivity of the individual diatom taxa from within the freshwater benthic community.

2. Materials and methods

2.1. Diatom collection locations

Diatoms were collected from Bluewater Creek (−19.14385, 146.26817) on the 18th of May 2012. The creek is located in North Queensland, Australia, at the base of Paluma State Forest near the town of Bluewater and is surrounded by eucalypt woodland. The stream substrate at the sample site is mostly large boulders, cobbles and pebbles, with a mean channel width of 7 m and highly diverse habitats present including deep and shallow pools, falls, runs and shallow riffles. The study site was chosen as there is no agriculture and only recreational activities occurring upstream of the site. The site is therefore considered a reference site for agricultural impacts such as herbicide pollution.

2.2. Sampling of natural benthic diatom communities

Pebbles and cobbles (approximately 5–25 cm in the longest axis) from the stream bed were chosen at random from various areas of a 50 m section of the stream bed and placed in trays for scrubbing. Multiple areas within a 50 m stretch of stream bed were sampled in order to include a variety of habitat types; riffles, pools and falls, for the purpose of obtaining the greatest possible number of taxa in a composite site collection. Areas which were stagnant pools and also very shallow areas likely to have been recently dried out were avoided to minimise collection of dead material. The benthic diatoms were removed from the rocks by scrubbing with a soft bristle toothbrush, using a squirt bottle with site water to wash off the detached material into a collection tray. The detached benthic diatoms were collected into a 500 mL plastic sample container as a composite sample, which was stored in the dark at site water temperature (21 ± 1 °C) for transportation to the lab.

2.3. Rapid toxicity tests

The benthic diatoms were exposed over 48 h to atrazine to determine the relative sensitivities of the taxa within the community. Tests were conducted in a controlled temperature laboratory at 24 ± 2 °C at a light intensity of 20 μmol m^{-2} s^{-1} (±10%), under a 12:12 hour light:dark cycle. After transportation to the lab the experiment was initiated within 4 h of sampling and included a 1 hour acclimatisation period to stabilise the temperature to that of the room.

The solution containing the removed benthic diatom community from Bluewater Creek was homogenised by gentle shaking and divided into 1 mL aliquots randomly assigned to 18 × 40 mL test vials by pipette. The test vials were then made up to a final volume of 20 mL with site water and spiked with a known atrazine herbicide concentration depending on treatment. The atrazine stock solution was prepared by dissolving analytical grade atrazine (Sigma Aldrich, CAS 1912-24-9) in site water using a carrier of 99% ethanol to increase the solubility of atrazine (2% *v:v*) with the maximum final volume of ethanol in the treatments being 0.05% (Magnusson et al., 2010). An ethanol control treatment with a final volume of 0.05% ethanol was included and compared to a site water only control after 48 h to eliminate carrier effects. All herbicide treatments were compared to the ethanol control. An additional control treatment at the start of the experiment ($t = 0$) was also prepared to indicate the diatom community and health at the start of the experiment. The experiment had a static water supply, without renewal of water or agitation for the duration of the test period as is common in algal bioassays (Larras et al., 2012; Magnusson et al., 2008). Diatoms were exposed to atrazine concentrations of 50, 200 and 500 μg L^{-1}, which were shown to elicit a response in the sensitive taxa from trial tests (data not shown) and are environmentally realistic in the region (Smith et al., 2012). All treatments and controls were replicated thrice.

Spiked water samples were also prepared in the same manner as each herbicide test treatment (50, 200, 500 μg L^{-1}) to be analysed for determination of the actual atrazine concentrations, which were within 15% of the nominal values (Supplementary Table S1).

2.4. Preservation of samples

After the exposure period (48 h) the contents of each replicate test vial were preserved with 3 drops of Lugol's iodine solution. The lids of the glass test vials were replaced and agitated to loosen the algae and ensure uniform preservation for later identification. After the preserved samples had settled, 10 mL of liquid was poured from each test vial, and the settled benthic diatoms were carefully transferred into a 10 mL sample storage container.

2.5. Identification of diatoms

Diatoms were identified by observation under an Olympus BX50 light microscope. Sub samples were taken from each replicate and observed in a Lund cell at a 400× magnification. Counting was conducted in random transects along the Lund cell until a total of at least 100 cells were counted and identified per replicate, which was sufficient for enumerating the common taxa in the sample; rare taxa that did not occur in every replicate were not included in analysis. Benthic diatoms were identified to the genus level using the following international (Cox, 1996; Round et al., 1990) and Australian (Gell, 1999; Sonneman et al., 2000) keys.

2.6. Health status of diatoms

The growth rates of the various diatoms differs substantially, and is very slow for some benthic taxa with doubling rates as low as 0.1–0.3 d^{-1}; this would make estimation of growth rate via cell counts difficult and lengthy (Admiraal, 1976; Gould and Gallagher, 1990). Therefore we have used a method of health classification similar to the live cell counts performed in other studies (Debenest et al., 2009; Pohlon et al., 2010; Proia et al., 2011), except data is recorded on a per taxon basis and includes identification of taxa as well as classification of health. The health status of the diatom cells was recorded as the number of diatom cells per genus that were either 'healthy' or 'unhealthy'. Cells were classified depending on the condition of the stained chloroplast. If it appeared more than 50% intact then it was classed as a healthy cell, and if the chloroplast was <50% intact or absent or the frustule was broken then it was classed as unhealthy (Supplementary Table S2). Broken frustules were only counted if more than 50% of the valve was left intact and could be identified. Diatom community composition was calculated using only the healthy cells in order to determine the effects on the live benthic community. The percentage of healthy cells in each treatment was calculated as a proportion of the total number of cells counted in that treatment per genus.

2.7. Statistical analysis

We assessed the effects of herbicide concentration on the health of diatoms using a generalised linear model (GLM). Concentration response of the diatom genera was performed using GLM on binary health data (healthy/unhealthy) with a logit link function. The model estimated the likelihood that a diatom cell would be healthy based on the concentration of exposure (50, 200, 500 μg L^{-1}) compared to the ethanol control and was carried out on a per taxon basis. Where atrazine exposure resulted in a significant decline in diatom cell health the EC50 was calculated with nominal concentrations using probit analysis (Finney, 1971).

The health of the cells was also assessed at the start ($t = 0$) and the end (48 h) of the experiment to insure the stability of control health and to eliminate any carrier effects. It was important to determine the background level of health for each genus, as this was expected to differ depending on the successional stage of the benthic diatom community at the time of collection (Davie et al., 2012). The background health of test controls (48 h) was assessed using GLM as described above, compared to the start of experiment controls ($t = 0$) as the reference parameter. Background health, concentration response and EC50 calculations were computed using SPSS 18 statistical package (SPSS 18).

Non-metric multi-dimensional scaling (MDS) ordination was conducted to examine community compositional changes of the healthy benthic diatom community among treatment groups at Bluewater Creek. MDS was conducted from the Bray Curtis index of similarity on untransformed community composition data. Only the community composition data for the healthy cells was used in the MDS for the common taxa (taxa which were observed at least once in every sample). A one way ANOSIM was used to determine the differences in the healthy diatom community between treatments. SIMPER analysis was performed to determine which taxa contributed to the differences between groups. Multivariate statistical analysis was performed using PRIMER v6 (Clarke and Gorley, 2006).

3. Results

3.1. Background health within control groups in rapid toxicity tests

The background health of diatoms remained relatively consistent between controls across most genera from Bluewater Creek throughout the experiment. No carrier effect was observed for any of the taxa in the study (Supplementary Table S3). There were no differences between health of cells at the start of the experiment ($t = 0$) and the ethanol controls (48 h) across all diatom genera (Supplementary Table S3).

3.2. Concentration response and relative sensitivity of the diatom genera

Differences in the relative atrazine sensitivity between benthic diatom genera were observed (Fig. 1). The most tolerant genera did not show a significant change in the health of cells with herbicide exposure: *Navicula*, *Eunotia* and *Achnanthidium* (Table 1). Diatoms from the genus *Navicula*, showed no concentration response to atrazine treatments and were the most tolerant in the benthic diatom community (Table 1 and Fig. 1a). The most sensitive diatom genera within the benthic community were *Gomphonema*, *Ulnaria*, *Cymbella* and *Amphora*, all of which showed a significant concentration response at the highest treatment of 500 μg L^{-1} (Table 1). This was equivalent to a decline relative to the control by 74% in *Gomphonema*, 62% in *Amphora*, 54% in *Cymbella* and 34% in *Ulnaria* (Table 1). *Gomphonema* (Fig. 1b) displayed a significant threshold concentration response to atrazine exposure and was the most sensitive taxa with an EC50 of 43.5 μg L^{-1} (Table 1). The genera *Ulnaria*, *Cymbella* and *Amphora* responded with significant dose–response relationships to atrazine exposure (Fig. 1c, e and f).

3.3. Community effects of herbicide exposure

The non-metric MDS ordination (stress = 0.09) showed a gradient of change in community composition of healthy benthic diatoms from the control groups to the highest herbicide exposure groups (Supplementary Figure S4). The separation of the highest concentration treatment is evident and the ANOSIM results were significant overall (Global $R = 0.361$, p-value = 0.005); however, the pairwise comparisons were not significant. The differences in community composition observed can be attributed to a decline in the most sensitive taxa and the increase of tolerant taxa after herbicide exposure (Table 1). The genera that had the greatest influence on the differences between the communities were *Amphora*, *Navicula* and *Ulnaria*, with each genus contributing approximately 19% to the Bray–Curtis dissimilarity between groups.

4. Discussion

A new method was established to determine the relative herbicide sensitivity of diatoms within a natural benthic community using rapid toxicity testing. The relative sensitivity of multiple diatom genera from a diverse field derived sample was determined from one 48 hour exposure test. This method is quicker and less costly than traditional methods of testing diatoms and algae which involve establishing cultures of each taxa and then testing each species individually (Brain et al., 2012; Larras et al., 2012; Magnusson et al., 2008, 2010; Moro et al., 2012; Nelson et al., 1999; Peterson et al., 1997; Tang et al., 1997). The method used in this study is based on the rapid toxicity approach previously used with invertebrates (Hickey et al., 2009; Kefford et al., 2005; Kefford et al., 2003). With the application of multiple rapid toxicity tests, herbicide sensitivity data for many taxa can be produced in a short period of time. This method is advantageous for the development of a traits based index, which would require sensitivity data for as many local taxa as possible.

We previously tested the use of an artificial substrate method for the collection of field derived natural benthic diatom communities (Guasch and Sabater, 1998; Laviale et al., 2011; Proia et al., 2011) for use in rapid toxicity tests. However, a number of sampling cages containing glass slides (Supplementary Figure S5) were lost or buried by substrate during the colonisation period due to the extremity of flow events in the study region, and the remaining substrates had highly variable densities of diatom growth. Another method using pebble substrates collected *in situ* was also tested. Unfortunately, we observed a very low density of diatoms on the small pebbles collected during the study, and since the purpose of retrieving pebbles from the field was to obtain a natural benthic community containing as many taxa as possible, this method was deemed unsuitable. Furthermore the diatom flora of small pebbles may only represent taxa that are rapid colonisers and may not reflect the general diatom community at a site due to the frequent movement and burial thereby resetting the colonisation process (Davie et al., 2012). These approaches were abandoned in favour of the scrubbing method of benthic diatom collection described in this study, which was quicker, requiring no prior site visits, and less expensive, requiring no specialised equipment. The results derived from this method showed limited variation of healthy cells in the controls over the test period (Supplementary Table S3), validating this method for comparisons

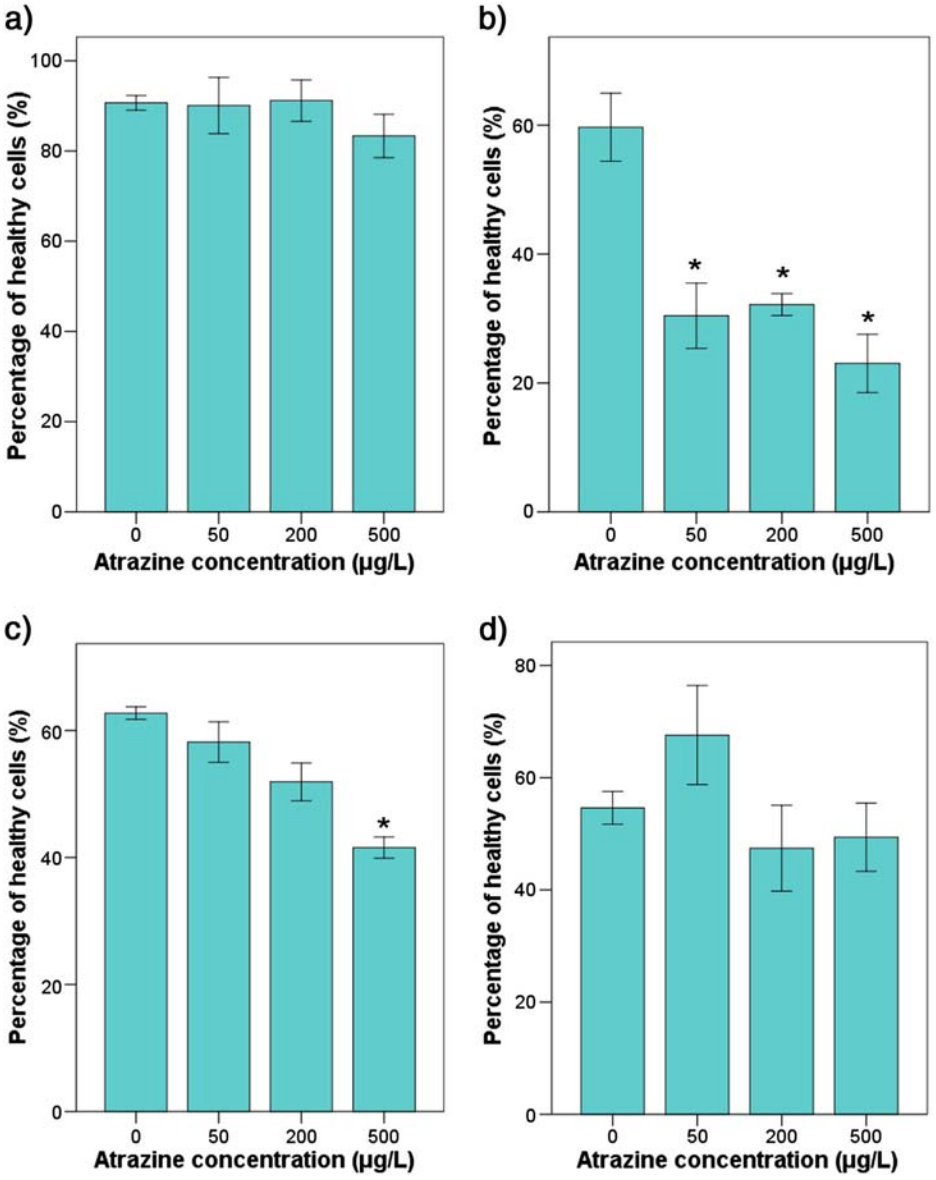

Fig. 1. Effects of atrazine on the health (%) of diatom cells by genus a) *Navicula*, b) *Gomphonema*, c) *Ulnaria*, d) *Achnanthidium*, e) *Cymbella*, f) *Amphora* and g) *Eunotia* at 48 h of exposure (Error bars represent ± 1 SE). Treatments marked * are statistically different from ethanol controls at alpha 0.05.

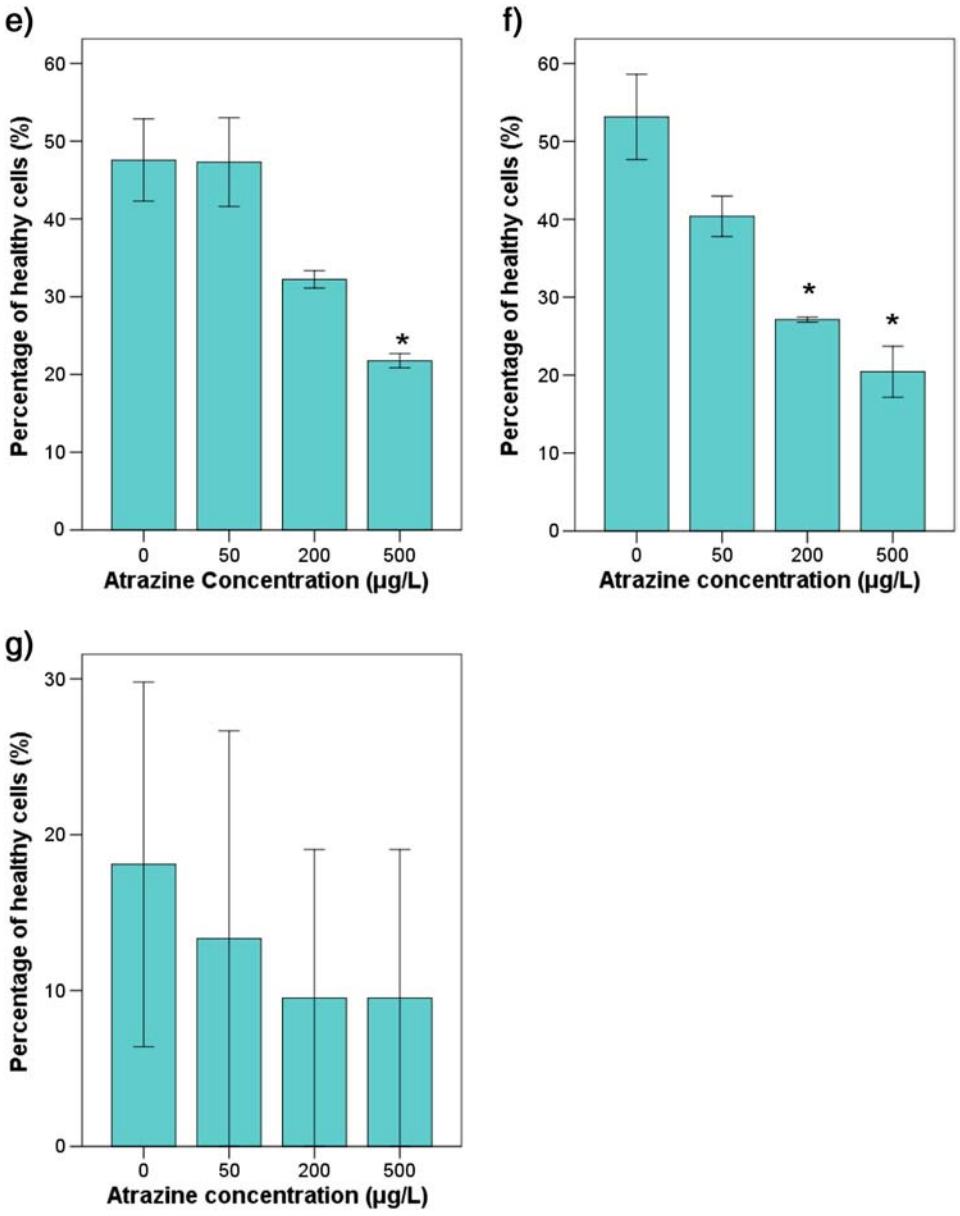

Fig. 1 (*continued*).

between treatments and controls and enabling the relative sensitivities of multiple taxa in a natural benthic diatom community to be determined from one 48 hour rapid test.

This study identified differences in the herbicide sensitivity of freshwater diatom genera within a natural benthic community. Identifying taxa by genus was necessary for the determination of cell health and to avoid uncertainty associated with identifying to the species level from live material. It is possible that that the individual species contributing to the genus tested here might not be representative of other members of the genus which were not tested, potentially leading to contradictory results. For example, Larras et al. (2012) found that *Gomphonema parvulum* was relatively tolerant to atrazine, whereas in this study *Gomphonema* was the most sensitive. However, a study by Growns (1999) found that genus and species level identification were similar at predicting impacts of river regulation because of the small number of species in a majority of diatom genera. Further studies should investigate whether this is the case for herbicide impacts and whether relative herbicide sensitivity differs between members of the same genera from within natural benthic communities.

In this study *Navicula* was the most tolerant genus to atrazine exposure and other genera such as *Ulnaria*, *Gomphonema*, *Cymbella* and *Amphora* were relatively more sensitive. *Navicula* are considered in the literature to be tolerant of both nutrient and herbicide pollution (Chalifour and Juneau, 2011; Guasch and Sabater, 1998). However, Magnusson et al. (2010) found that photosynthetic inhibition occurred in *Navicula sp.* at atrazine concentrations much lower than the exposures in this study. The concentrations of atrazine in the current study (50–500 µg L^{-1}) exceed the field measured peak concentrations which regularly reach 10 µg L^{-1} in rivers that flow into the Great Barrier Reef (GBR) (Brodie et al., 2012; Lewis et al., 2012; Smith et al., 2012). However, considering that PSII herbicides such as atrazine often occur in mixtures of two or more and that their toxicity is additive (Magnusson et al., 2010), recent studies have calculated the atrazine equivalent concentrations of PSII herbicide mixtures to be up to 807 µg L^{-1} at sites within the GBR catchment area (Smith et al., 2012), justifying the ecological relevance of these results in identifying which taxa are most at risk of herbicide toxicity in field derived communities.

Table 1
Concentration response of the diatom genera using the generalised linear model (GLM). Effects of herbicide concentration on the health of diatom cells at each treatment level (50, 200, 500 μg L^{-1} atrazine) at 48 h of exposure compared to ethanol controls (no herbicide). Percentage of healthy cells per treatment, percentage composition of the healthy benthic diatom community, EC50 and EC10 values. – Not calculable.

Genus	Concentration (μg L^{-1})	Sig.	Healthy cells (% ± SE)	Community composition (%)	EC50 (μg L^{-1})	EC10 (μg L^{-1})
Navicula	0	–	90.67 ± 1.62	13.12 ± 3.21	–	–
	50	0.808	90.08 ± 6.23	17.19 ± 0.91		
	200	0.905	91.17 ± 4.59	17.21 ± 0.86		
	500	0.403	83.33 ± 4.81	17.75 ± 2.06		
Ulnaria	0	–	62.74 ± 0.99	39.81 ± 1.57	1241	84.12
	50	0.434	58.18 ± 3.19	39.73 ± 1.31		
	200	0.059	51.92 ± 2.99	42.22 ± 2.82		
	500	0.000	41.56 ± 1.68	42.84 ± 1.33		
Gomphonema	0	–	55.03 ± 6.88	14.35 ± 2.03	43.47	0.123
	50	0.013	24.17 ± 6.14	10.44 ± 0.92		
	200	0.010	23.89 ± 2.00	11.08 ± 0.73		
	500	0.001	14.54 ± 2.76	9.18 ± 1.20		
Achnanthidium	0	–	54.62 ± 2.91	8.50 ± 0.99	–	–
	50	0.437	67.59 ± 8.83	10.18 ± 1.08		
	200	0.429	47.43 ± 7.64	11.79 ± 1.80		
	500	0.500	49.38 ± 6.08	16.23 ± 1.86		
Eunotia	0	–	18.10 ± 11.70	1.85 ± 1.31	–	–
	50	0.367	13.33 ± 13.33	0.97 ± 0.97		
	200	0.485	9.52 ± 9.52	1.13 ± 1.13		
	500	0.485	9.52 ± 9.52	1.19 ± 1.19		
Cymbella	0	–	47.57 ± 5.29	9.20 ± 1.29	419.1	75.26
	50	0.995	47.31 ± 5.70	7.51 ± 0.59		
	200	0.193	32.22 ± 1.11	6.78 ± 0.52		
	500	0.027	21.77 ± .92	4.63 ± 0.39		
Amphora	0	–	53.13 ± 5.47	13.17 ± 2.88	241.8	14.29
	50	0.119	40.38 ± 2.59	13.98 ± 3.10		
	200	0.002	27.12 ± .34	9.79 ± 1.20		
	500	0.000	20.44 ± 3.27	8.17 ± 1.63		

Open questions include: (1) whether the diatom cells which appeared healthy were physiologically impaired and (2) whether those individuals regarded as unhealthy would recover following the cessation of herbicide exposure. Prosser et al. (2013) observed rapid recovery of quantum yield from periphyton communities after the cessation of atrazine exposure with ≥95% recovery within 48 h and other studies have observed similar rapid recovery in quantum yield (Brain et al., 2012; Laviale et al., 2011). However, as those studies did not investigate changes in cell health, it is uncertain what relevance they have for the recovery of diatoms classified as unhealthy in the current study. Indeed other studies (Dorigo et al., 2010; Magnusson et al., 2012) have observed much slower recovery of the periphyton community structure following exposure to herbicides in the field (Morin et al., 2010). Such studies suggest that alternative approaches, for example the changes in cell health used in the current study, should also be investigated. Indeed, the ability of certain diatoms to recover after herbicide exposure may be an important trait for consideration alongside sensitivity in the development of a traits-based monitoring index using diatoms (Gustavson et al., 2003). The results of the relative sensitivity by diatom genera are meant as a means for ranking the relative sensitivities of the taxa or for classifying their sensitivity (e.g. sensitive or tolerant) and not as an indication of what atrazine concentration will or will not harm diatom taxa in nature where exposure periods might be different and might co-occur with other stressors.

5. Conclusions

The current study developed a new method of producing sensitivity data for a range of individual diatom taxa from within a natural benthic community in a short period of time. The rapid toxicity tests provided consistent control data with a low variability in the health of cells per genera at the start of the experiment, which was suitable for determining the differences in the relative sensitivity of diatom genera to atrazine exposure. This method can deliver sensitivity data for multiple taxa from the one 48 hour test, without the need for cultures or multiple site visits, and will be useful for the production of herbicide sensitivity data that can be used for a new traits based index that can detect herbicide toxicity using benthic diatoms. These results could also be used to make species sensitivity distributions (SSDs) based on communities of diatoms that occur in specific regions. We thus recommend the use of this method for conducting rapid toxicity testing of diatoms. Future studies should investigate the differences in sensitivity between members of the same genera from within natural benthic communities, the effects of herbicidal mode of action on relative sensitivity and the effects of light on PSII sensitivity in freshwater diatoms.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.03.115. This data includes measured concentrations of atrazine, pictures of healthy vs. unhealthy diatoms from each genus, and extended statistical results.

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